

Pattern formation by competition: a biological example

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Abstract

We present a simple model based on a reaction-diffusion equation to explain pattern formation in a multicellular bacterium (*Streptomyces*). We assume competition for resources as the basic mechanism that leads to pattern formation; in particular we are able to reproduce the spatial pattern formed by bacterial aerial mycelium in case of growth in minimal (low resources) and maximal (large resources) culture media.

1 Introduction

Bacteria are unicellular organisms generally studied as isolated units, however they are interactive organisms able to perform collective behaviour, and a clear marker of the presence of a multicellular organization level is the formation of growth patterns [1, 2]. Particularly it has been pointed out that unfavorable conditions may lead bacteria to a cooperative behavior, as a means to react to the environmental constraints [3].

Many studies about the multicellular level of organization of bacteria have been proposed and pattern formation during colonies growth has been observed in Cyanobacteria [1], in *Bacillus subtilis* [1, 4, 5], in *Escherichia coli* [1, 6], *Proteus mirabilis* [1, 2] and others. Some of these patterns have been studied by mathematical models [3, 4, 5, 6, 7], that explain the macroscopic patterns through the microscopic observations.

There is a group of bacteria that differs from those cited above because their normal morphological organization is clearly multicellular: Actinomycetes, and *Streptomyces* is a genus of this group. *Streptomyces* are gram-positive bacteria that grow as mycelial filaments in the soil, whose mature colonies may contain two types of mycelia, the substrate, or vegetative, mycelium and the aerial mycelium, that have different biological roles [8]. Vegetative mycelium absorbs the nutrients, and is composed of a dense and complex network of hyphae usually embedded in the soil. Once the cell culture becomes nutrient-limited, the aerial mycelium develops from the surface of the vegetative mycelium. The role of this type of mycelium is mainly reproductive, indeed the aerial mycelium develops the spores and put them in a favorable position to be dispersed [8, 9].

In our laboratory we have isolated a bacterial strain, identified with morphological criteria as belonging to *Streptomyces*. This strain is interesting because its growth pattern differs on maximal and minimal

culture media. On maximal culture medium (LB, Luria Broth) [10], after 3 – 4 days of growth at $30^{\circ}C$, the strain shows a typical bacterial growth with formation of the rounded colony characteristic of most of the bacterial strains (Fig. 1) [8]. On minimal culture medium (Fahreus) [11] growth proceeds more slowly than in maximal media and a concentric rings pattern of aerial mycelium sets up (Fig. 2). The rings are centered on the first cell that sets up the colony - we call it the founder - where usually the aerial mycelium develops as well. The number of rings increases with time till 7 – 8 after 20 days of growth at $30^{\circ}C$. In both cases agar concentration was 1.5%.

The presence of concentric rings patterns is a quite common feature in bacterial and fungi colonies [12]; many models can originate such patterns [14], a possible explanation was proposed in [13], where is suggested that the interplay of front propagation and Turing instability can lead to concentric ring and spot patterns. A different approach based on competition for resources has been recently proposed [15, 16] to study species formation as pattern formation in the genotypic space. We consider a similar mechanism to investigate the spatial pattern formations observed in our laboratory in a *Streptomyces* colony.

2 The model

2.1 Biological constraints

Before introducing the mathematical model we have to go through some of the biological features of the system. Aerial mycelia are connected through the vegetative hyphae network. This network has a peculiar structure in the *Streptomyces* isolated in our laboratory, indeed we observe that the growing boundary of the substrate mycelium is made by many hyphae extending radially from the founder so that, in this area, the substrate mycelium has a radial polarity, also if the hyphae have many branching segments.

Substrate mycelium has the biological objective to find nutrients to give rise to spores, therefore we expect that on minimal media a strong competition arises for the energetic resources between neighbor substrate mycelia, whereas in maximal media, where there are sufficient nutrients, the competition is weaker.

If the cells are connected mainly along the radial direction, then competition will be stronger along this direction than along the tangential one. In other words, in the growing edge of the colony, the competition is not isotropic but, following the vegetative mycelium morphology, it will be stronger among cells belonging to neighboring circumferences (radial direction) than among cells of the same (tangential direction), and we will keep track of these aspects in the model. Although the radial polarity is lost inside the colony, the asymptotic distribution of aerial mycelium is strongly affected by the initial spots derived by the growing boundary of the vegetative mycelium.

Finally another important feature of the biological system is the presence of a founder. The founder behaves as every other aerial mycelium - it competes with the other cell -, moreover it is the center of every circle. That means that every hypha originates from the founder: it is the source of the vegetative hyphae, and as the colony grows the ring near the founder become increasingly densely packed. Moreover during the enlargement of the colony no new center sets up and therefore substrate mycelium density is highest near the founder and decreases radially away from it.

To summarize, in our model we make the following assumptions based on the previous considerations.

- There is competition among every aerial mycelium for some substances that we assume for sake of simplicity uniformly distributed over the culture.
- We consider only the aerial mycelium: we do not introduce explicitly the substrate mycelium but we take in account it assuming that
 - a) The competition is stronger along the radial direction than along the tangential one.
 - b) The probability for the aerial mycelium to appear is higher near the founder

Assuming this framework we show that a concentric rings pattern may be explained as a consequence of strong competition, and a rounded pattern of weak competition. From the biological point of view this result implies that the formation of concentric rings patterns is a mean that *Streptomyces* adopts to control growth.

2.2 The mathematical model

In the following we propose a mathematical model to reproduce the aerial mycelium growth patterns described in the Introduction. This model is derived from a similar model introduced, in a different framework, (species formation in genotypic space) in [15, 16].

Let us consider a two-dimensional spatial lattice, that represents the Petri dish. Each point \mathbf{x} is identified by two coordinates $\mathbf{x} = (x_1, x_2)$, we study the temporal evolution of the normalized probability $p(\mathbf{x}, t)$ to have an aerial mycelium in \mathbf{x} position at time t . The evolution equation for $p(\mathbf{x}, t)$, is in the form:

$$p(\mathbf{x}, t + 1) = A(\mathbf{x}, p(\mathbf{x}, t))p(\mathbf{x}, t) \quad (1)$$

where $A(\mathbf{x}, p(\mathbf{x}, t))$ is the probability of formation of a new aerial mycelium in position \mathbf{x} and we suppose it can depend also on the distribution $p(\mathbf{x}, t)$. According to the hypothesis described above, it is the product of two independent terms:

$$A(\mathbf{x}, p(\mathbf{x}, t)) = \frac{A_1(\mathbf{x})A_2(\mathbf{x}, p(\mathbf{x}, t))}{\bar{A}}$$

where $A_1(\mathbf{x})$ is the so-called static fitness, and represents the probability of growth of an aerial mycelium in presence of an infinite amount of resources (no competition). The founder is the source of every hypha, so we expect it will be a decreasing function of the distance $|x|$ from the founder, with $|x| = \sqrt{x_1^2 + x_2^2}$, assuming the founder occupies $(0, 0)$ position.

The second term $A_2(\mathbf{x}, p(\mathbf{x}, t))$ is the competition term, and in general it depends on the whole spatial distribution $p(\mathbf{x}, t)$, moreover we suppose that two aerial micelia compete as stronger as close they are.

\bar{A} is the average fitness and it is necessary to have $p(\mathbf{x}, t + 1)$ normalized. It is defined as following :

$$\bar{A}(t) = \int_{\mathbf{x}} A(\mathbf{x}, p(\mathbf{x}, t))d\mathbf{x}$$

Both terms are positive, therefore can be written in the exponential form

$$A_1(\mathbf{x})A_2(\mathbf{x}, p(\mathbf{x}, t)) = \exp \left(H_1(\mathbf{x}) - J \int_{\mathbf{y}} K(d(\mathbf{x}, \mathbf{y}))p(\mathbf{y}, t)dy \right)$$

where J is the intensity of competition (it will be large in presence of strong competition, i.e. low resource level) and $K(d(\mathbf{x}, \mathbf{y}))$ is a decreasing function of the distance between two mycelia $d(\mathbf{x}, \mathbf{y})$.

We also allow $p(\mathbf{x}, t)$ to diffuse to the nearest neighbors with diffusing coefficient μ^1 .

Finally we get:

$$p(\mathbf{x}, t + 1) = \frac{\exp \left(H_1(\mathbf{x}) - J \int_{\mathbf{y}} K(d(\mathbf{x}, \mathbf{y}))p(\mathbf{y}, t)dy \right)}{\bar{A}(t)} p(\mathbf{x}, t) + \mu \nabla^2 p(\mathbf{x}, t) \quad (2)$$

According to the assumptions stated in Section 2.1, we now introduce the particular forms for $H_1(\mathbf{x})$ and $K(d)$. $H_1(\mathbf{x})$ depends on the distance from the founder $H_1(\mathbf{x}) = H_1(|x|)$, and the competition kernel $K(d)$, depending on the distance d between mycelia. As mentioned above, we expected the probability

¹The presence of diffusion is necessary to allow the bacteria to populate the whole lattice

of growth for the aerial mycelium to be higher near the founder, therefore $H_1(|x|)$ has to be a decreasing function of $|x|$. For the sake of simplicity we have chosen a single maximum, “almost linear” function,

$$H_1(|x|) = h_0 + b \left(1 - \frac{|x|}{r} - \frac{1}{1 + |x|/r} \right) \quad (3)$$

that has a quadratic maximum in $\mathbf{x} = (0, 0)$ (founder), in fact close to $\mathbf{x} = (0, 0)$ we have $h(|x|) \simeq h_0 - b|x|^2/r^2$ and for $|x| \rightarrow \infty$, is linear $h(|x|) \simeq h_0 + b(1 - |x|/r)$. b and r control the intensity of the static fitness.

The competition kernel $K(d)$ has to be a steep decreasing function of d ; we expect to have a finite range of competition, i.e. two mycelia at distance $d > R$ do not compete (or compete very weakly). A possible choice is:

$$K(d) = \exp \left(-\frac{1}{4} \left| \frac{d}{R} \right|^4 \right) \quad (4)$$

We have also chosen the form for the kernel (4) and static fitness (3) because it is possible to derive some analytical results [16] that assure us the existence of a non-trivial spatial distribution for exponential kernel with exponent greater than 2; R is the range of competition. All the numerical and analytical results described in this paper are obtained using (3, 4), but we have also tested similar potential obtaining the same qualitative results.

Computing numerically from Eq. (2) the asymptotic probability distribution $p(\mathbf{x}) \equiv p(\mathbf{x}, t)_{t \rightarrow \infty}$, we get, for different values of the parameters, two types of spatial patterns. In particular numerical and analytical studies (see Ref. [16]) show that the crucial parameter is $G = (J/R) / (b/r)$, i.e. the ratio between the intensity of competition and the intensity of the static fitness.

For small values of G , that is the competition is rather weak or in other words we have a maximal medium, we get a single peak gaussian-like distribution centered on the founder (similar to the one showed on the left in Fig. 5 (left) with $G = 0.5$).

For larger values of G we get a multi-peaked distribution (see Fig. 3, $G = 248.0$), where the central peak (founder) is still present, but we get also some others peaks at an approximate distance R , range of competition, between each other. This is the expected pattern for an isotropic competition, in fact the presence of equally distanced spots is due to the competition term, that inhibits the growth of any aerial mycelium around another one.

To obtain spatial patterns similar to the concentric rings observed in our experiments, some feature of the peculiar spatial structure of *Streptomyces* has to be added. As stated before, we hypothesize that due to the presence of the substrate mycelium morphology the competition is much stronger in the radial direction (along the hyphae) than in the tangential direction.

Therefore we decompose the distance between any points \mathbf{x} and \mathbf{y} in a radial $d_R(\mathbf{x}, \mathbf{y})$ and tangential part $d_T(\mathbf{x}, \mathbf{y})^2$ (see Fig. 4)

$$d(\mathbf{x}, \mathbf{y})^2 = d_R(\mathbf{x}, \mathbf{y})^2 + \alpha d_T(\mathbf{x}, \mathbf{y})^2 \quad (5)$$

where α is a parameter that allows to change the metric of our space.

For $\alpha > 1$ the relative weight of tangential distance is larger than one due to the lack of cell communications along this direction, the competition is mainly radial along the hyphae because the mycelia do not compete if they are not directly connected by an hypha. For $\alpha = 1$ we get the usual euclidean distance.

Using the distance (5) in Eq.(2) with $\alpha > 1$ and strong competition we are able to obtain a set of rings composed by equally spaced spots at fixed distances from the founder (see Fig. 5 (right) for $\alpha = 6$), while in presence of large resource we still have a single peaked distribution (Fig. 5 (left)). For larger

values of α the rings become continuous, while for low values, $\alpha \rightarrow 1$, the multi-peaked structure of $p(\mathbf{x})$ appears.

These results are in agreement with those presented in Ref. [16], where an one-dimensional system is considered. In this case the genotypic space plays the role of the real space, and using and a gaussian kernel

$$K(d) = \exp\left(-\frac{1}{2}\left|\frac{d}{R}\right|^2\right)$$

is possible to derive analytically the value of transition G_c between the two regimes (single peaked and multi-peaked distribution). It is, for $\mu \rightarrow 0$ (slow diffusion) and $\frac{r}{R} \rightarrow 0$ (static fitness almost flat)

$$G_c\left(\frac{r}{R}\right) \simeq G_c(0) - \frac{r}{R}$$

with $G_c(0) = 2.216\dots$. Thus for $G > G_c\left(\frac{r}{R}\right)$ we have a multi-peaked distribution, while for $G < G_c\left(\frac{r}{R}\right)$ only the fittest one survives (single-peaked distribution).

3 Discussion and conclusions

We isolated a strain of *Streptomyces* that has a dual pattern of growth concerning the aerial mycelium: it gives rise to concentric rings centered on the founder cell, or to the classic circular bacterial colony. The medium is discriminant: in minimal media the first type of pattern arises, in maximal media the second one.

The substrate mycelium follows a different pattern: optical microscopy observations revealed that every hypha originates from the primordial central colony (the founder). Moreover the growth of the substrate mycelium growing edge proceeds in radial direction from the founder.

Using a simple mathematical model for the formation of aerial mycelium we are able to simulate both aerial mycelium spatial patterns. The parameter we modulate to obtain these two different patterns is the competition intensity. Indeed the main assumption of the model is that there is competition among the hyphae of vegetative mycelia for the energetic sources necessary for the formation of the aerial mycelium. In a medium with low nutrient concentration there is a strong competition for the aerial mycelium formation - and the model produces concentric rings patterns - instead in a maximal medium the competition is weaker - and the model produces the classic circular bacterial colony.

The aerial mycelium is derived by the substrate mycelium, so we derived the constraints of the model from the morphological observations concerning the substrate mycelium described in the Introduction. The system has a radial geometry centered on the founder (the probability of formation of aerial mycelium is higher near the founder), and we assumed that the competition is affected by this feature. Indeed the competition is stronger along an hypha due to the cell-cell communication typical of the “multicellular” organization of *Streptomyces*. This implies that the competition is stronger along the radial direction than along the tangential, at least in the outer boundary of the colony.

The growth pattern description above is referred to the presence of one single primordial colony. In presence of two or more colonies close one another we have observed different patterns with additive and negative interactions among the colonies. Our minimal model is not able to reproduce these behaviors, due to the fact that in presence of many founders the simple assumptions of radial growth centered on a single founder is no more fulfilled.

In conclusion we have found some peculiar spatial patterns for the aerial mycelium of *Streptomyces*. We have proposed a simple mathematical model to explain these patterns assuming competition along the hyphae as the main ingredient that leads to pattern formation. Our numerical results are able to reproduce spatial patterns obtained experimentally under different conditions (minimal and maximal medium), while to get more complex behavior (interference patterns, see Fig. 6) we expect more “chemical” species have to be added to our minimal model.

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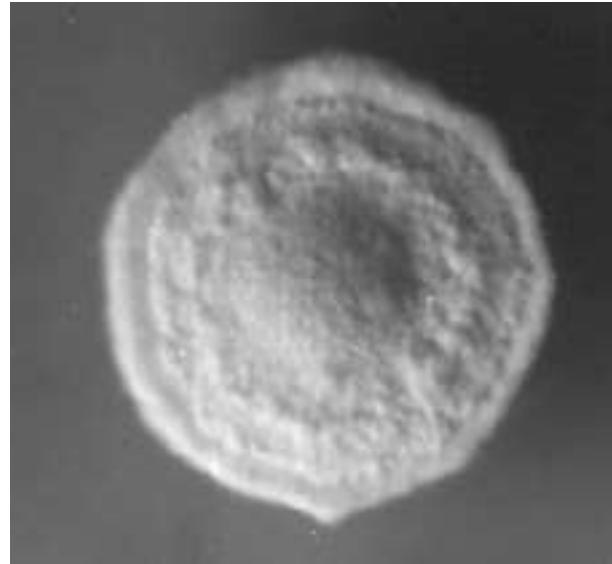


Figure 1: Pattern formed by *Streptomyces* growing in maximal culture media. See details in the text.

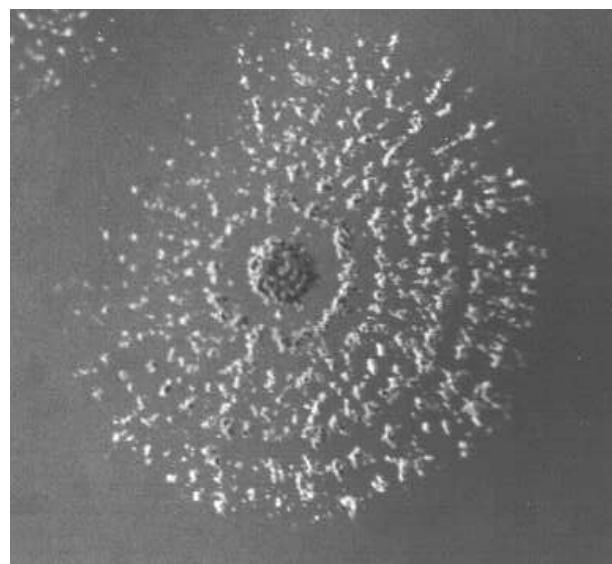


Figure 2: Pattern formed by *Streptomyces* growing in minimal culture media. See details in the text.

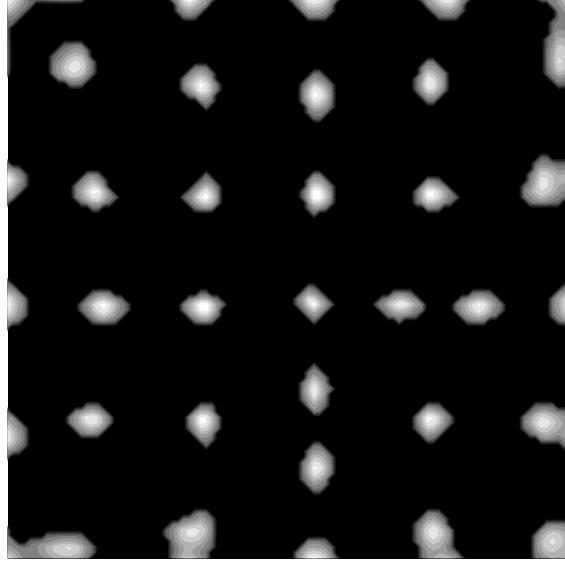


Figure 3: Asymptotic distribution $p(\mathbf{x})$ for isotropic competition ($\alpha = 1$) plotted in inverse gray-scale, i.e. black $p(\mathbf{x}) = 0$, white $p(\mathbf{x}) = 1$, in low resource case: $\mu = 0.015$, $h_0 = 0$, $b = 0.05$, $r = 2$, $J = 56.0$ and $R = 9$. The discretization of space (square lattice) for numerical solution of Eq. 2) is clearly evident.

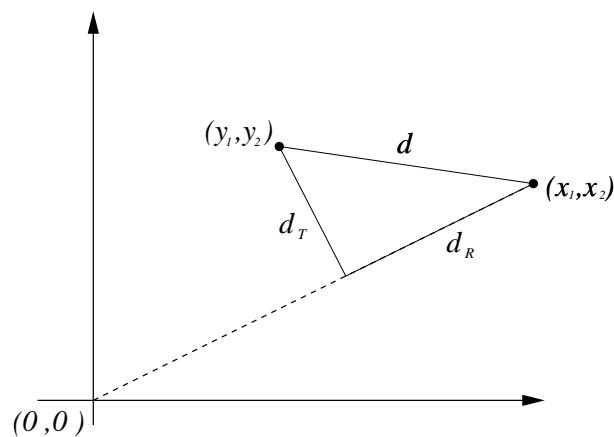


Figure 4: Decomposition of the distance between any points \mathbf{x} and \mathbf{y} in a radial $d_R(\mathbf{x}, \mathbf{y})$ and tangential part $d_T(\mathbf{x}, \mathbf{y})^2$ with respect to a circle centered in the founder placed in $(0, 0)$.

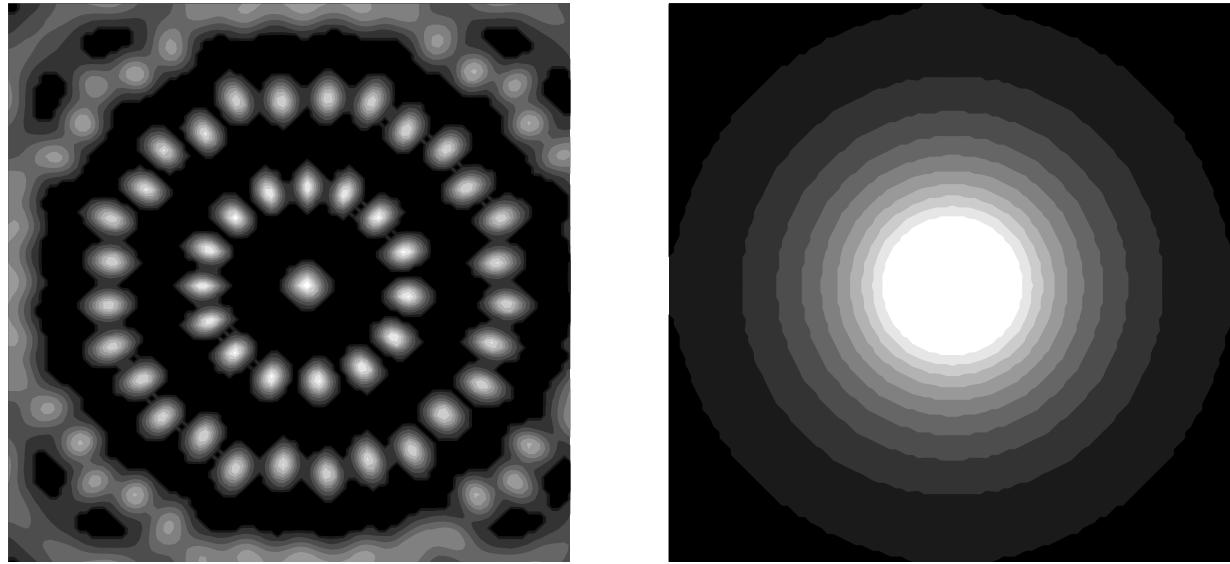


Figure 5: Asymptotic distribution $p(\mathbf{x})$ for different values of parameters, plotted in inverse gray-scale, i.e. black $p(\mathbf{x}) = 0$, white $p(\mathbf{x}) = 1$. Left (large resources): $\mu = 0.015$, $h_0 = 0$, $b = 0.1$, $r = 2$, $J = 0.1$ and $R = 4$. Right (low resources): $\mu = 0.015$, $h_0 = 0$, $b = 0.05$, $r = 2$, $J = 56.0$ and $R = 9$.

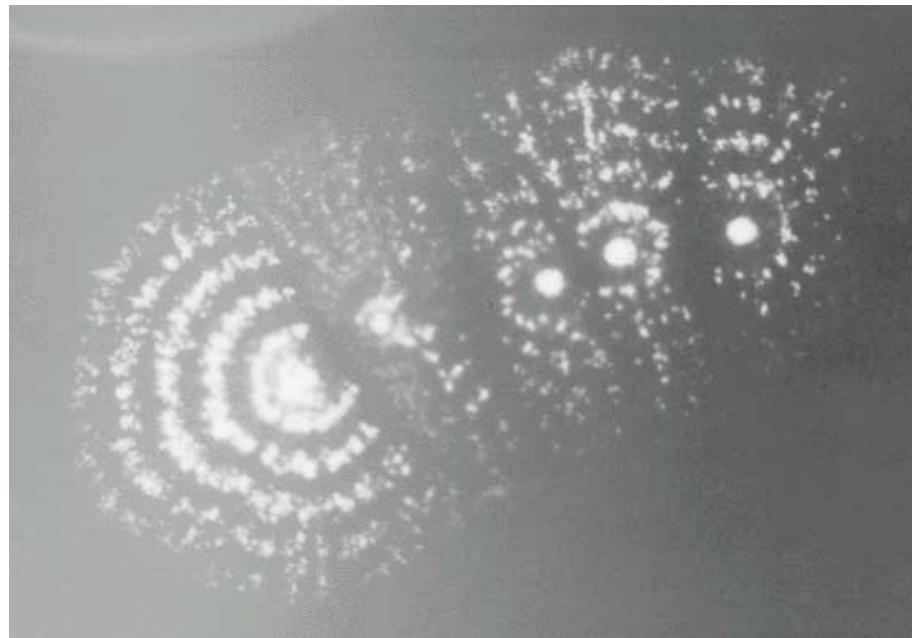


Figure 6: Interference pattern formed by *Streptomyces* colonies growing in minimal culture media.